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Vancomycin-resistant *Enterococcus faecium* circulating in a Portuguese hospital (2009-2021) challenges classical infection control

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Abstract

Background: Vancomycin-resistant *Enterococcus faecium* (VREfm) prevalence greatly varies in European countries yet continues to rise [1]. We aimed to characterize clinical-VREfm from a local hospital (800-beds) during 2009-2021. **Methods:** VREfm [n=175; mostly from urine (40%)] were collected from medical-47% and surgical-32% wards. Antibiotic-resistance (ABR) and occurrence of *vanA/vanB*, plasmids [rep-pLG1/rep-pRUM/rep-Inc18(rep1/rep2)] and virulence genes were screened by disk-diffusion/broth-microdilution (EUCAST/CLSI) and PCR, respectively. Representative VREfm/year (n=81) were selected for MLST+WGS (Illumina-NovaSeq) and analysed through CGE-tools (homemade virulence and bacteriocins(bac) databases) and cgMLST/Ridom-SeqSphere^{+}. **Results:** All isolates were resistant to glycopeptides [98%-*vanA*;2%-*vanB*], ampicillin, ciprofloxacin, erythromycin [*erm*(B)/*msr*(C)], less to gentamicin (49%;*aac(6')-Ie-aph(2'')-Ia*), tetracycline [27%; *tet*(M)/*tet*(L)], quinupristin-dalfopristin (5%) and/or linezolid (2%;GT2576 mutation). VREfm were oligoclonal (until 7 STs/year), mostly clustering into ST117-31%, ST78-19% and ST80-12%. For each ST, different complex-types (CT) were detected each year, but some were identified in different wards from 3-months (ST412-CT258/ST117-CT4659) to 2-4-years (ST117-CT24/CT6602/CT6603; ST78-CT330). Sequenced VREfm showed slight overlap (ST117-CT24 and ST78-CT230) with other global Efm. Recent years (>2019) concentrated the majority of novel CTs, without common clones over time, and ST494 (associated with a German outbreak) was introduced. ABR and virulence patterns were similar throughout the years. Bac-profiles (13 patterns) were similar between common STs/CTs. One new bacteriocin was detected >2017, Bac43 increased over time and BacAS9 was exclusively detected in ST117. The typical VREfm plasmidome [RepA_N-pRUM/pLG1-like, Inc18, Rep3-pB82/pEF418, Rep_Trans-pEFNP1/pRI1] was observed across the years however, Rep-pRUM decreased. A novel RepA_N-replicase (69% nucleotide identity with pRUM) was identified in most VREfm during 2009-2021. *In silico* analysis showed its occurrence in ~100 Efm-genomes/GenBank from different countries after 2000. **Conclusion:** Current clinical VREfm display a strong oligoclonal nature. Nosocomial transmission events and/or import of VREfm from colonized patients may explain the long-term persistence of particular clones. The continuous flux between community and hospital clones may feed the emergence of new lineages/opportunities for adaption hampering classical infection control interventions.

Keywords: vancomycin resistance; *Enterococcus faecium*; clones; bacteriocins; nosocomial infections

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